Resistance of Sweetpotato Genotypes to Adult *Diabrotica* Beetles

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ABSTRACT Production of sweetpotatoes, *Ipomoea batatas* (L.) Lam. (Convolvulaceae), is limited by several insect pests, including *Diabrotica* spp. (Coleoptera: Chrysomelidae), and new integrated pest management (IPM) techniques for this crop are needed. Host plant resistance is one attractive approach that fits well into IPM programs. A host plant resistance research program typically depends on reliable bioassay procedures to streamline evaluation of germplasm. Thus, a bioassay technique was developed for evaluating sweetpotato germplasm by using adults of the banded cucumber beetle, *Diabrotica balteata* LeConte, and spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber. A single beetle was placed on a piece of sweetpotato peel (periderm and cortex with stele removed) that was embedded periderm-side up in plaster in a petri dish. Feeding and longevity of insects on 30 sweetpotato genotypes were evaluated in two experiments by using this procedure. Adult longevity ranged from 7 to 11 d for starved individuals to 211 d for beetles fed a dry artificial diet. Longevity of banded cucumber beetles that fed on sweetpotato peels ranged from 12 d for the most-resistant genotype to 123 d for SC1149-19, a susceptible control cultivar. Longevity of spotted cucumber beetles was slightly shorter than longevity of banded cucumber beetles. For the most resistant sweetpotato genotypes, both *Diabrotica* species exhibited a significant delay in initiation of feeding, and more beetles died on these genotypes before they had fed. Both antibiosis and nonpreference (antixenosis) are important mechanisms of resistance in sweetpotato genotypes. This bioassay was consistent with field results, indicating that this technique could be useful for evaluating resistance to *Diabrotica* spp. in sweetpotato genotypes.

KEY WORDS plant resistance, *Ipomoea*, *Diabrotica*, bioassay

Production of sweetpotatoes, *Ipomoea batatas* (L.) Lam. (Convolvulaceae), is severely limited by several insect pests (Cuthbert 1967, Schalk and Jones 1985). Control of these pests with insecticides can be expensive and unreliable, and it may cause environmental or safety concerns. Thus, there is a need for integrated pest management (IPM) techniques that reduce the impact of pesticides in sweetpotato production (Jackson et al. 2002a). Host plant resistance is one such insect control technique that can result in a more environmentally friendly IPM approach. Pest resistance to insects can be the cornerstone of a successful IPM program.

The genus *Diabrotica* (Coleoptera: Chrysomelidae) has a wide host range, including sweetpotatoes (Cuthbert 1967, Saba 1970, Chalfant et al. 1990). The banded cucumber beetle, *Diabrotica balteata* LeConte, and the spotted cucumber beetle (or southern corn rootworm), *Diabrotica undecimpunctata howardi* Barber, are the most important *Diabrotica* pests of sweetpotatoes in the Western Hemisphere (McKinlay 1992, Capinera 2001). The spotted cucumber beetle is a more widespread pest than the banded cucumber beetle that is found in the southern United States (Pitre and Kantack 1962, Jackson et al. 2005). Because insects are seldom found when sweetpotato roots are dug, it is difficult to determine whether root injury was caused by wireworms (W), cucumber beetles (*Diabrotica* sp. [D]), or flea beetles (*Systena* sp. [S]), so the damage by these coleopteran pests is often lumped into a single category called WDS (Cuthbert and Davis 1971, Schalk et al. 1991). Observed WDS damage could be due to one or more of these pests, including a combination of cucumber beetle species.

Cuthbert and Jones (1972) first showed that the level of resistance to the WDS complex could be increased after only four generations of recurrent selection in randomly crossing populations of sweetpotato genotypes. However, breeding for pest resistance is a difficult, complex, and time-consuming procedure for sweetpotato that is a clonally propagated hexaploid (Jones et al. 1986). Most sweetpotato breeding programs use a mass selection technique with a polycross nursery of up to 25 parental genotypes, and because pollination is done by natural bee populations, only the female parent is known for new

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seedlings (Jones et al. 1986). Also, sweetpotatoes are clonally propagated so no further improvements can be made to existing cultivars or new genotypes. Despite these difficulties, several new sweetpotato varieties and advanced breeding lines with resistance to the WDS complex have been developed and released by the USDA–ARS (Schalk et al. 1991; Collins and Hall 1992; Bohac et al. 2000, 2002; Bohac and Jackson 2005). Antibiosis, nonpreference (antixenosis), and tolerance are all important mechanisms in pest resistance in sweetpotatoes (Barlow and Rolston 1981). For example, several antibiotic components from the periderm and cortex of insect-resistant sweetpotato genotypes have been identified (Cuthbert and Davis 1971; Schalk et al. 1986a; Peterson et al. 1998, 2003, 2005; Jackson and Peterson 2000; Harrison et al. 2003a,b).

A host plant resistance research program typically depends on reliable bioassay procedures to streamline evaluation of germplasm (Smith et al. 1994). Also, a well-designed bioassay can be critical to advancing an understanding of the mechanisms of host plant resistance to insects in a particular crop. Thus, for the study described herein, a laboratory bioassay was developed for evaluating feeding damage by adult cucumber beetles on storage roots of sweetpotato genotypes.

Materials and Methods

Laboratory colonies of the spotted cucumber beetle and the banded cucumber beetle were originally started from adults collected from cucurbit fields at the U.S. Vegetable Laboratory (USVL), Charleston, SC. Colonies have been maintained continuously for several years at the USVL by using rearing procedures adapted from Schalk (1986) and Schalk and Peterson (1990). These procedures require germinating wheat sprouts to support the first two instars, but the remainder of the larval development is on a commercial artificial diet (wheat germ and casein base with no antibiotics) (product no. F9760B, Bio-Serv, Frenchtown, NJ). Adults also were fed this dry artificial diet with added soy flour (Pollen Substitute, Walter T. Kelley Co., Clarkson, KY). The laboratory colonies were last infused with over 100 field-collected adults in 2002.

Experiment 1 (2001–2003). Seventeen sweetpotato genotypes with a diverse genetic background were evaluated in the first experiment. They were three insect-susceptible, orange-fleshed cultivars ('Beauregard', 'Jewel', and 'SC1149-19'); five insect-resistant, orange-fleshed cultivars ('Carolina Bunch', 'Excel', 'Patriot', 'Regal', and 'Ruddy'); two dry-fleshed cultivars ('Liberty' and 'Sumor'); three plant introductions from National Plant Germplasm System ('Camote Morado', 'Simon No. 1', and 'Tinian') (USDA 2005); and four advanced genotypes (95-161, W-274, W-364, and W-376). Eleven of the sweetpotato entries were from the USDA–ARS breeding program, and they were developed using recurrent or mass selection techniques (Jones 1965, Jones et al. 1986). Controls consisted of treatments in which cucumber beetles were 1) starved and denied access to water, 2) starved but provided water from a damp cotton role, 3) fed stele from SC1149-19, and 4) fed the dry artificial diet used in colony rearing.

Camote Morado (PI 399163), Excel, Patriot, Regal, Ruddy, Sumor, and Tinian have high levels of resistance to the WDS complex in the field (Jones et al. 1985, 1989; Dukes et al. 1987, Bohac et al. 2000, 2002; Jackson et al. 2002b,d, 2003; Jackson and Bohac 2003). Carolina Bunch and Liberty are reported to have low-to-moderate levels of resistance to the WDS complex (Dukes et al. 1992, Bohac et al. 2003, Jackson et al. 2003, LaBonte 2005). Beauregard, Jewel, and SC1149-19 are susceptible to most soil insect pests, including WDS (Pope et al. 1971; Rolston et al. 1987; Collins and Hall 1992; Thompson et al. 2001; Jackson et al. 2002a,b,c,d, 2003; Jackson and Bohac 2003, 2004).

Storage roots used for these experiments were grown in field plots in Bamberg and Charleston counties, SC, 2000–2003, by using standard production practices, except that no insecticides were used. After harvest, roots were cured at ≈45°C for 7–10 d then stored at 15–17°C until needed for bioassays. Undamaged roots of each genotype were selected for bioassays. While being careful not to scratch the periderm, whole sweetpotato roots were gently washed with tap water and allowed to air dry. Pieces of unblemished sweetpotato peel (defined here as the periderm and cortex with stele removed) were then cut from the sweetpotato roots by using a sharp knife. The thickness of periderm and cortex vary among sweetpotato genotypes (Schalk et al. 1986b), so care was taken to ensure that the periderm was not damaged and that no stele portion of the sweetpotato root was left attached to the peel samples that measured 1–4 cm^2 in area. The sweetpotato peels were gently washed, soaked for 30 min in a 1.5% solution of captan (Captan Fungicide 50 WP, Southern Agricultural Insecticides, Inc., Palmetto, FL) to prevent growth of fungi, and then they were allowed to air dry. Preliminary bioassay experiments showed that this rate of Captan had no measurable effects on survival or feeding behavior of cucumber beetle adults.

For the adult bioassay, pieces of sweetpotato peel that totaled ≈4 cm^2 were embedded periderm-side up in wet DAP Plaster Wall Patch (DAP Products Inc., Baltimore, MD) in 10-cm-diameter plastic petri dishes (Tyco Healthcare Group, Mansfield, MA). Care was taken to ensure that only periderm was exposed and that the edges of the cut pieces were completely covered by the wet plaster, which was then allowed to dry. Thus, the insects were forced to initially feed through the unblemished periderm before reaching the cortex of the sweetpotato peel.

A single adult *Diabrotica* beetle (sex not determined) was placed on the piece of sweetpotato periderm in each petri dish. The beetles were deprived of food for 24 h before the start of the experiment. Water was provided with saturated cotton wicks placed on the plaster surface away from the root pieces. The petri dishes were incubated at ≈24°C. Petri dishes were examined for feeding holes, frass, and dead beetles every day for the first 2 wk and three times a week

thereafter. Adult longevity was recorded as the time (in days) from the start of the experiment until the beetle was found dead. The date for the initiation of feeding was recorded when feeding holes or new frass were present. About every 2 wk, after the sweetpotato peels had been eaten, dried out, or deteriorated, surviving adults were switched to a fresh piece of sweetpotato peel in a new petri dish.

This experiment was repeated seven times for *D. balteata* and nine times for *D. undecimpunctata*, and there were three replications each time the experiment was repeated. Thus, data for *D. balteata* were from 21 individual adults for each sweetpotato genotype, and data for *D. undecimpunctata* were from 27 individual adults. Data for longevity, initiation of feeding, and percentage of adults that died before they fed were subjected to analysis of variance (ANOVA), and means were separated by Fisher least significant difference (LSD) at the 5% probability level (PROC GLM, SAS Institute 1989).

Experiment 2 (2004–2005). A different group of 17 sweetpotato genotypes was evaluated in the second experiment. This set of sweetpotato entries had a more narrow genetic background than the genotypes in the first experiment. Insect-susceptible Beauregard and SC1149-19 and an insect-resistant Regal were included as controls. All of the sweetpotato genotypes except Beauregard were from the USDA–ARS breeding program, and the advanced genotypes ("W" lines) were selected because of their known resistance to soil insect pests in field trials (Jackson et al. 1999, 2002a,b,c,d, 2003; Jackson and Bohac 2003, 2004). Storage roots used for these experiments were grown in field plots in Bamberg and Charleston counties, SC, in 2003 by using standard production practices, except that no insecticides were used. Bioassay procedures for the second experiment were identical to the first. This experiment was repeated six times for each *Diabrotica* species, and there were three replications each time the experiment was repeated. Thus, data for each insect species were from 18 individual adults for each genotype. Longevity data were subjected to ANOVA, and means were separated by Fisher LSD at the 5% probability level (PROC GLM, SAS Institute 1989).

Field Evaluations (1997–2004). The seventeen sweetpotato entries from the first bioassay experiment were grown in 30 replicated field experiments at the USVL during 1997–2004. Each sweetpotato entry was planted in three to four replications of single row, 10-plant plots arranged in a randomized complete block experimental design. Local production practices were followed, except that no insecticides were applied. When rainfall was not adequate during the growing season, supplemental overhead irrigation was applied. Plots were harvested in the fall when roots had reached maturity.

After harvest, all individual roots were scored for insect damage by previously published procedures (Schalk et al. 1991; Lawrence et al. 1999; Jackson et al. 1999, 2002a). The severity index for the WDS complex was calculated by averaging the rating given to each root (1, one to five holes or scars; 2, six to 10 holes or scars; 4, >10 holes or scars). Because there were significant yearly fluctuations in the levels of WDS injury and because not every sweetpotato genotype was grown in each experiment, WDS severity index data were weighted by multiplying each data point by a weighting factor calculated as a proportion of the average WDS rating for that experiment against the average over all experiments during the 8 yr of evaluations. A combined data set for all sweetpotato genotypes from the first bioassay experiment was subjected to ANOVA, and means were separated by Fisher LSD at the 5% probability level (PROC GLM, SAS Institute 1989). Average WDS severity indices for each sweetpotato genotype were correlated to longevity data from the first bioassay experiment by using linear regression analysis (PROC REG, SAS Institute 1989).

Results and Discussion

ANOVA indicated that there were highly significant treatment effects for overall longevity of adult *Diabrotica* beetles in the first experiment (*D. balteata:* $F = 15.9$; df = 20, 399; $P < 0.0001$ and *D. undecimpunctata:* $F = 17.8$; df = 20, 502; $P < 0.0001$) (column 2 of Tables 1 and 2). As expected, starved insects, either with or without supplemental water, died first (7.0–10.4 d after the start of the test). Conversely, adults fed artificial diet or the stele from SC1149-19 lived an average of over 20 wk for banded cucumber beetles (Fig. 1). Maximum longevity was 473 d for a *D. balteata* beetle, and 329 d for a *D. undecimpunctata* adult, both on SC1149-19 stele.

Beetles on many of the sweetpotato genotypes in the first experiment had significantly shorter life spans than beetles on the artificial diet, SC1149-19 stele, or SC1149-19 peel, and in many cases longevity was not significantly different than for the starved controls (column 2 of Tables 1 and 2). For several of these same genotypes, there was a significant delay in the initiation of feeding compared with beetles on the artificial diet and SC1149–19 stele (*D. balteata:* $F = 2.68$; df = 20, 245; $P < 0.0002$ and *D. undecimpunctata:* $F = 2.24$; df = 20, 399; $P < 0.0018$) (column 3 of Tables 1 and 2). Also, for some of the resistant genotypes, a significantly higher percentage of beetles died before they had fed compared with beetles on the artificial diet or SC1149-19 stele (*D. balteata:* $F = 1.89$; df = 20, 245; $P < 0.0139$ and *D. undecimpunctata:* $F = 2.28$; df = 20, 399; $P < 0.0015$) (column 4 of Tables 1 and 2). When longevity was recalculated using only the adults that had fed (minus those who starved before initiating feeding), ANOVA indicated that there still were highly significant treatment effects (*D. balteata:* $F = 12.1$; df = 20, 330; $P < 0.0001$ and *D. undecimpunctata:* $F = 10.78$; df = 20, 351; $P < 0.0001$) (column 5 of Tables 1 and 2). Both species of beetles on the peels of Ruddy, Excel, 95-161, Tinian, and W-376 had a significant delay in the initiation of feeding, a longevity not significantly different from the starved controls, and a significantly higher percentage of adults that died before they tried to feed compared with beetles on the artificial diet or stele of SC1149-19 (Tables 1 and 2).

Table 1. For banded cucumber beetles, average overall longevity, delay time before initiation of feeding, average percentage of adults that died before feeding, and longevity of adults that fed on the peels of 17 sweetpotato genotypes (experiment 1), Charleston, SC, 2001–2004

Treatment	Overall longevity (d ± SE)	Delay before[a] feeding (d ± SE)	% adults that died before feeding (± SE)	Longevity of adults that fed (d ± SE)
Artificial diet	211.0 ± 21.0a	0.00 ± 0.00e	0.0 ± 0.0d	211.0 ± 21.0a
SC1149-19[b] (PI 634401[c]) stele	183.5 ± 21.9a	0.00 ± 0.00e	0.0 ± 0.0d	183.5 ± 21.9a
SC1149-19[b] (PI 634401[c]) peel	116.7 ± 25.4b	0.47 ± 0.35cde	6.7 ± 6.7cd	122.6 ± 26.0b
Beauregard (PI 566613[c]) peel	63.0 ± 18.9c	0.53 ± 0.29cde	0.0 ± 0.0d	63.0 ± 18.9cd
W-274[b] peel	62.4 ± 22.5c	0.67 ± 0.39cde	6.7 ± 6.7cd	65.6 ± 22.6c
Jewel (PI 531122[c]) peel	54.0 ± 14.9cd	0.93 ± 0.56cde	6.7 ± 6.7cd	56.7 ± 15.5cde
W-364[b] Peel	45.9 ± 14.4cde	0.73 ± 0.41cde	0.0 ± 0.0d	45.9 ± 14.4cde
Sumor[b] (W-201) (PI 566657[c]) peel	42.6 ± 16.0cde	2.20 ± 0.63bcd	13.3 ± 9.1bcd	46.7 ± 17.4cde
Camote Morado (PI 399163[c]) peel	36.8 ± 11.3cde	2.33 ± 1.00abc	13.3 ± 9.1bcd	40.0 ± 12.3cde
Simon No. 1 (PI 508513[c]) peel	32.5 ± 12.2cde	0.33 ± 0.33de	0.0 ± 0.0d	32.5 ± 12.2cde
Regal[b] (W-152) (PI 566650[c]) peel	32.2 ± 12.4cde	1.60 ± 0.86bcde	6.7 ± 6.7cd	33.7 ± 13.0cde
Patriot[b] (W-244) peel	31.5 ± 8.0cde	1.17 ± 0.44bcde	11.1 ± 7.6bcd	34.1 ± 8.5cde
Liberty[b] (W-341) peel	31.3 ± 13.1cde	0.53 ± 0.29cde	0.0 ± 0.0d	31.3 ± 13.1cde
Carolina Bunch[b] (W-241) peel	27.5 ± 8.7cde	0.50 ± 0.27cde	7.1 ± 7.1cd	29.0 ± 9.0cde
Ruddy[b] (W-287) peel	16.5 ± 2.1de	2.13 ± 0.88bcd	26.7 ± 11.8abc	18.8 ± 2.4de
Excel[b] (PI 566625[c]) peel	15.6 ± 2.4de	2.13 ± 0.93bcd	26.7 ± 11.8abc	17.6 ± 2.7de
95–161[b] peel	14.3 ± 4.1de	3.00 ± 1.53ab	33.3 ± 23.3ab	11.5 ± 3.4e
Tinian (PI 153655[c]) peel	13.5 ± 2.6e	1.47 ± 0.82bcde	20.0 ± 10.7abcd	14.8 ± 2.9e
W-376[b] peel	10.6 ± 1.0e	4.20 ± 0.82a	40.0 ± 13.1a	11.9 ± 1.2e
Starved with water	7.0 ± 0.4e			
Starved without water	7.3 ± 0.7e			

Means within columns followed by a common letter are not significantly different (LSD; $P = 0.05$) (SAS Institute 1989).
[a] Average delay in time that adult beetles started feeding compared with the artificial diet control (time zero).
[b] Sweetpotato genotype from the USDA–ARS breeding program at the U.S. Vegetable Laboratory, Charleston, SC.
[c] Plant Introduction no., National Plant Germplasm System, USDA–ARS, Griffin, GA (http://www.ars-grin.gov/cgi-bin/npgs/html/tax_site_acc.pl?S9%20Ipomoea%20batatas%20var.%20batatas) (USDA 2005).

ANOVA indicated that for the second experiment there also were highly significant treatment effects for overall longevity of adult *Diabrotica* beetles (*D. balteata:* $F = 52.8$; df = 20, 377; $P < 0.0001$ and experiment 2, *D. undecimpunctata:* $F = 27.3$; df = 20, 355; $P < 0.0001$) (Table 3). For the second experiment where there was a narrower genetic base, there were fewer differences in average longevity among the advanced

Table 2. For spotted cucumber beetles, average overall longevity, delay time before initiation of feeding, average percentage of adults that died before feeding, and longevity of adults that fed on the peels of 17 sweetpotato genotypes (experiment 1), Charleston, SC, 2001–2004

Treatment	Overall longevity (d ± SE)	Delay before[a] feeding (d ± SE)	% adults that died before feeding (± SE)	Longevity of adults that fed (d ± SE)
Artificial diet	153.8 ± 14.8a	0.00 ± 0.00d	0.0 ± 0.0e	153.8 ± 14.8a
SC1149-19[b] (PI 634401[c]) stele	146.0 ± 11.3a	0.00 ± 0.00d	0.0 ± 0.0e	146.0 ± 11.3a
Beauregard (PI 566613[c]) peel	68.6 ± 15.7b	1.71 ± 0.56abcd	16.7 ± 7.8cde	79.5 ± 17.6b
SC1149-19[b] (PI 634401[c]) peel	67.8 ± 14.6b	0.71 ± 0.34cd	12.5 ± 6.9de	75.3 ± 15.8b
Regal[b] (W-152) (PI 566650[c]) peel	54.7 ± 14.7bc	2.29 ± 0.70abc	25.0 ± 10.1abcde	66.8 ± 18.1bc
W-364[b] peel	53.0 ± 11.5bc	2.08 ± 0.65abc	16.7 ± 7.8cde	61.0 ± 12.8bcd
Jewel (PI 531122[c]) peel	46.8 ± 12.7bcd	1.25 ± 0.52bcd	25.0 ± 9.0abcde	58.0 ± 15.5bcde
Carolina Bunch[b] (W-241) peel	41.3 ± 11.3bcde	1.92 ± 0.76abcd	25.0 ± 9.0abcde	49.7 ± 14.0bcdef
Patriot[b] (W-244) peel	37.3 ± 9.5cdef	3.13 ± 0.92ab	20.8 ± 8.5bcde	43.1 ± 11.3bcdef
Camote Morado (PI 399163[c]) peel	27.7 ± 7.1cdefg	3.56 ± 1.18a	22.2 ± 10.1bcde	31.7 ± 8.5cdef
Liberty[b] (W-341) peel	24.5 ± 7.7defg	1.33 ± 0.47bcd	16.7 ± 7.8cde	27.5 ± 8.2def
W-376[b] peel	23.9 ± 7.5defg	3.08 ± 0.78ab	37.5 ± 10.1abcd	31.8 ± 10.8cdef
W-274[b] peel	19.6 ± 6.5defg	2.08 ± 0.69abc	33.3 ± 9.8abcd	23.7 ± 9.1def
Excel[b] (PI 566625[c]) peel	17.0 ± 3.7efg	3.00 ± 0.73ab	37.5 ± 10.1abcd	20.3 ± 5.4ef
95–161[b] peel	14.5 ± 6.4efg	2.33 ± 1.08abc	50.0 ± 15.1a	20.4 ± 10.4ef
Ruddy[b] (W-287) peel	14.4 ± 1.9efg	3.54 ± 0.88a	41.7 ± 10.3abc	17.5 ± 2.6f
Simon No. 1 (PI 508513[c]) peel	14.0 ± 3.4efg	0.50 ± 0.50cd	33.3 ± 21.1abcd	16.1 ± 4.1f
Tinian (PI 153655[c]) peel	11.9 ± 1.2fg	1.88 ± 0.67abcd	33.3 ± 9.8abcd	13.4 ± 1.5f
Sumor[b] (W-201) (PI 566657[c]) peel	11.6 ± 1.2fg	2.29 ± 0.58abc	45.8 ± 10.4ab	14.0 ± 1.8f
Starved with water	10.9 ± 1.1fg			
Starved without water	7.4 ± 0.5g			

Means within columns followed by a common letter are not significantly different (LSD; $P = 0.05$) (SAS Institute 1989).
[a] Average delay in time that adult beetles started feeding compared with the artificial diet control (time zero).
[b] Sweetpotato genotype from the USDA–ARS breeding program at the U.S. Vegetable Laboratory, Charleston, SC.
[c] Plant Introduction no., National Plant Germplasm System, USDA–ARS, Griffin, GA (http://www.ars-grin.gov/cgi-bin/npgs/html/tax_site_acc.pl?S9%20Ipomoea%20batatas%20var.%20batatas) (USDA 2005).

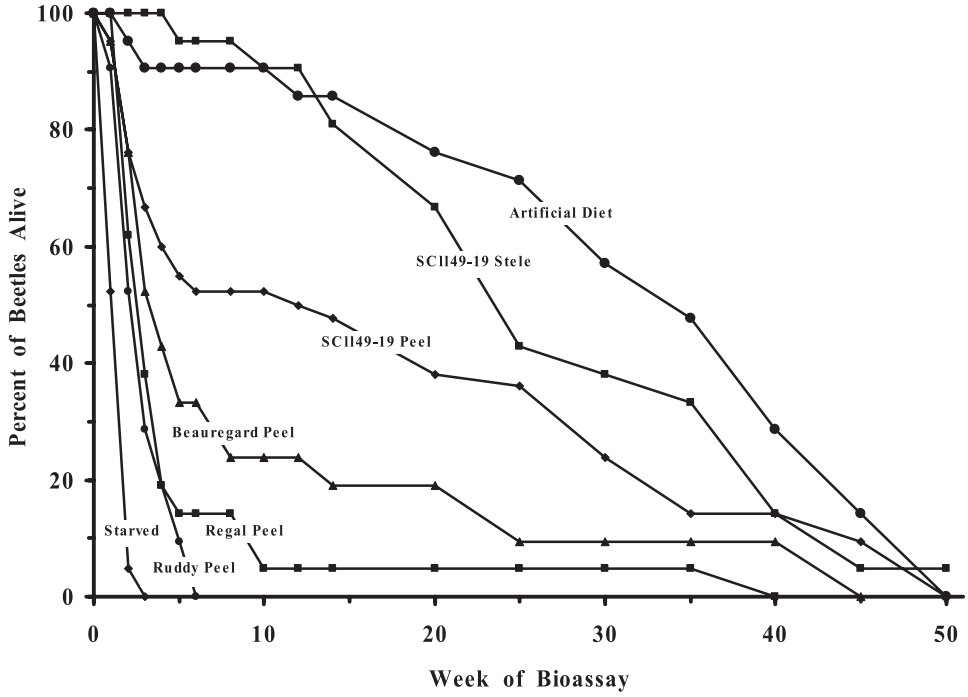

Fig. 1. Percentage of adult banded cucumber beetles surviving over time on seven treatments of a feeding bioassay during 2001–2004 (experiment 1).

genotypes (W lines) from the USDA–ARS breeding program. This indicates that the source of resistance for *Diabrotica* species is consistently present throughout these USVL genotypes. These data show that this technique could be used to verify that resistance factors have been transferred to new sweetpotato genotypes, which is an essential element in a sweetpotato breeding program.

Table 3. Average longevity (± SE) of adult banded or spotted cucumber beetles fed the peels of 17 sweetpotato genotypes (experiment 2), Charleston, SC, 2004–2005

Treatment[a]	Longevity (d ± SE)	
	Banded	Spotted
SC1149-19 stele	187.9 ± 18.9a	130.1 ± 17.0a
Artificial diet	174.8 ± 21.5a	97.8 ± 14.0b
SC1149-19 peel	50.0 ± 7.2b	35.2 ± 5.9cd
Beauregard peel	34.7 ± 7.2bc	45.6 ± 6.8c
W-386 peel	22.3 ± 2.4cd	27.3 ± 3.2de
Liberty (W-341) peel	21.6 ± 3.0cd	22.5 ± 3.4def
W-387 peel	21.3 ± 4.8cd	20.6 ± 3.7defg
W-385 peel	18.6 ± 1.6cd	18.4 ± 1.9efg
W-383 peel	16.4 ± 1.4cd	17.7 ± 1.9efg
W-392 peel	16.3 ± 1.4cd	16.9 ± 2.6efg
W-328 peel	16.0 ± 1.8cd	18.9 ± 2.3efg
W-381 peel	15.8 ± 1.7cd	19.4 ± 4.2efg
W-382 peel	15.8 ± 1.4cd	21.3 ± 3.4defg
Regal peel	15.1 ± 2.3d	20.8 ± 2.0defg
W-375[b] peel	14.9 ± 1.2d	16.4 ± 1.7efg
W-361 peel	13.4 ± 1.2d	13.6 ± 1.7efg
W-380 peel	13.2 ± 0.9d	17.8 ± 1.8efg
W-393 peel	13.2 ± 0.8d	22.4 ± 3.1defg
W-384 peel	12.4 ± 1.3d	19.6 ± 2.7defg
Starved with water	8.4 ± 1.0d	8.3 ± 0.9fg
Starved without water	6.8 ± 0.7d	6.7 ± 0.7g

Means within columns followed by a common letter are not significantly different (LSD; $P = 0.05$) (SAS Institute 1989).

[a] All sweetpotato genotypes except Beauregard from the USDA–ARS breeding program at the U.S. Vegetable Laboratory, Charleston, SC.

[b] W-375 was recently released as 'Charleston Scarlet' (Bohac and Jackson 2005).

Data from the first experiment suggest that both nonpreference and antibiosis mechanisms are involved in the resistance of sweetpotato genotypes evaluated in this study. Nonpreference is indicated by the significant proportion of individuals that never initiated feeding and by the delay in feeding by other beetles on the skin of many of the resistant sweetpotato genotypes. Antibiosis also seems to be a factor, because the longevity of beetles that did feed on resistant genotypes was significantly shorter than beetles that fed on the artificial diet and stele controls. This was not unexpected, because antibiosis components have been reported from the periderm and cortex of several of the insect-resistant sweetpotato genotypes used in this study (Peterson et al. 1998, 2003, 2005; Jackson and Peterson 2000; Harrison et al. 2003a,b).

ANOVA indicated that there were highly significant treatment effects among sweetpotato genotypes for WDS severity index in the field ($F = 63.0$; df = 16, 567; $P < 0.0001$) (Table 4). These measurements of WDS injury, which encompass damage by cucumber beetle larvae, were consistent with results of the adult bioassay. Linear regression analyses showed that average WDS severity indices were significantly correlated to longevity results for both species in the first bioassay experiment (*D. balteata:* $r^2 = 0.642$, $n = 16$, $P < 0.0001$ and *D. undecimpunctata:* $r^2 = 0.576$, $n = 16$, $P < 0.0004$), indicating that these techniques could be use-

Table 4. Average WDS severity index (+ SE) for 17 sweetpotato genotypes (Experiment 1) from field trials, Charleston, SC, 1997–2004

Sweetpotato genotype	WDS index (± SE)[a]
SC1149-19	1.437 ± 0.046a
Beauregard	1.063 ± 0.048b
Carolina Bunch	0.817 ± 0.063c
W-274	0.761 ± 0.051c
Jewel	0.707 ± 0.047cd
W-364	0.629 ± 0.093cde
Excel	0.551 ± 0.066def
Liberty	0.491 ± 0.046efg
Patriot	0.484 ± 0.073efg
Regal	0.402 ± 0.029fgh
W-376	0.343 ± 0.051hij
Ruddy	0.329 ± 0.080hij
Sumor	0.272 ± 0.024hij
95–161	0.224 ± 0.056hij
Tinian	0.213 ± 0.025hij
Simon No. 1	0.161 ± 0.088ij
Camote Morado (PI 399163)	0.084 ± 0.020j

Means within columns followed by a common letter are not significantly different (LSD; $P = 0.05$) (SAS Institute 1989).

[a] Average from 30 field experiments, 1997–2004, at the U.S. Vegetable Laboratory, Charleston, SC. WDS severity index: 1, one to five scars; 2, six to 10 scars; 4, >10 scars, averaged over all roots; maximum score, 4.0.

ful for evaluating pest resistance in sweetpotato genotypes to *Diabrotica* species. Regression analysis was not run on the second experiment because of the narrow genetic diversity of these sweetpotato genotypes and clumped distribution of WDS severity indices. These data suggest that even though it is larvae not adults of *Diabrotica* spp. that attack sweetpotato storage roots in the field, there seems to be a correlation between resistance responses to these life stages. *Diabrotica* adults are much easier to work with than larvae, and this bioassay procedure requires less labor than field evaluations.

Although Cuthbert and Jones (1972) first showed that the level of resistance to the WDS complex could be increased incrementally using recurrent selection, they did not attempt to simultaneously select for other desirable characteristics. Progress toward improved sweetpotato cultivars can only be made through a diligent and simultaneous evaluation procedure for all desirable characteristics. Therefore, as part of this procedure, each new sweetpotato genotype must be evaluated to see whether it has retained its resistance to insect pests. The results of the current study demonstrate that a laboratory bioassay with adult *Diabrotica* beetles could be used as part of this evaluation process. A disadvantage to this bioassay procedure as it was conducted in this study is that adults lived so long in the control treatments. However, this bioassay could be adapted so that survival data were collected after a set period of time. Survival of adults on the insect-resistant genotypes declines rapidly, and by 4 wk after initiation of the first experiment fewer than 25% of *D. balteata* beetles on peels of Ruddy or Regal were living (Fig. 1). However, >50% of the insects on the susceptible cultivars SC1149-19 and Beauregard were living after 4 wk, and this parameter could be used as a measure of resistance for sweetpotato germplasm.

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